MDR1 gene polymorphisms and disposition of the P-glycoprotein substrate fexofenadine

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Aims The C3435T polymorphism in the human *MDR1* gene is associated with lower intestinal P-glycoprotein expression, reduced protein function in peripheral blood cells and higher plasma concentrations of the P-glycoprotein substrate digoxin. Using fexofenadine, a known P-glycoprotein substrate, the hypothesis was tested whether this polymorphism also affects the disposition of other drugs in humans.

Methods Ten Caucasian subjects homozygous for the wild-type allele at position 3435 (CC) and 10 individuals homozygous for T at position 3435 participated in this study. A single oral dose of 180 mg fexofenadine HCl was administered. Plasma and urine concentrations of fexofenadine were measured up to 72 h using a sensitive LC/MS method. In addition, P-glycoprotein function was assessed using efflux of the P-glycoprotein substrate rhodamine 123 from $CD56^+$ cells.

Results Fexofenadine plasma concentrations varied considerably among the study population. However, fexofenadine disposition was not significantly different between the CC and TT groups (e.g. AUC$(0,\infty)$ CC *vs* TT: 3567.1 ± 1535.5 *vs* 3910.1 ± 1894.8 ng ml^{-1} h, NS; 95% CI on the difference -1364.9, 2050.9). In contrast, P-glycoprotein function was significantly decreased in $CD56^+$ cells of the TT compared with the CC group (rhodamine fluorescence CC *vs* TT: $45.6 \pm 7.2\%$ *vs* $61.1 \pm 12.3\%$, $P < 0.05$; 95% CI on the difference 5.6, 25.5).

Conclusions In spite of *MDR1* genotype-dependent differences in P-glycoprotein function in peripheral blood cells, there was no association of the C3435T polymorphism with the disposition of the P-glycoprotein substrate fexofenadine in this German Caucasian study population. These data indicate that other mechanisms including uptake transporter function are likely to play a role in fexofenadine disposition.

Keywords: fexofenadine, MDR1, P-glycoprotein, polymorphism, rhodamine 123

Introduction

The *MDR1* gene product P-glycoprotein (ABCB1) is an integral membrane efflux protein, which actively translocates its substrates from the inside of cells to their outside [1–3]. It was first discovered in cancer cells, where it contributes to the development of multidrug resistance (MDR) during chemotherapy of tumours [e.g. with vinca alkaloids, etoposide; for a review see Gottesman *et al.* [2]]. However, it is also expressed in normal tissues with an excretory function such as intestine, liver and kidneys and in the blood–brain barrier [4, 5]. It is now well established that intestinal P-glycoprotein located at the apical membrane of enterocytes pumps xenobiotics back into the gut lumen [6, 7] thereby limiting the absorption of orally administered P-glycoprotein substrates such as cyclosporin, HIV protease inhibitors, talinolol and digoxin [8–12]. Modification of P-glycoprotein function by

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co-administered drugs has recently been identified as a mechanism leading to increased or decreased plasma concentrations of P-glycoprotein substrates. The underlying mechanism of increased digoxin plasma concentrations during concomitant treatment with the antiarrhythmic quinidine is inhibition of P-glycoprotein-mediated elimination of the former by the latter [13]. In contrast, decreased digoxin or talinolol plasma concentrations during treatment with rifampicin were primarily due to induction of intestinal P-glycoprotein [10, 11].

In addition to these environmental factors, a polymorphism in exon 26 of the human *MDR1* gene (C3435T) was recently described, which was associated with a significantly reduced intestinal P-glycoprotein content in subjects with the TT genotype in comparison to subjects homozygous for the wild-type allele [14]. Accordingly, subjects with the TT genotype had higher steady-state plasma concentrations after oral administration of digoxin in comparison to wild-type subjects [14]. Reduced P-glycoprotein activity was also found in natural killer cells from healthy individuals having the TT genotype at position 3435 in comparison to subjects homozygous for the wild-type genotype [15]. Whether this polymorphism is also associated with altered pharmacokinetics of other P-glycoprotein substrates has not been studied. Like digoxin, the H_1-receptor antagonist fexofenadine, which is used for treatment of seasonal allergic rhinitis and chronic idiopathic urticaria, is a P-glycoprotein substrate, which is metabolized only to a minimal extent in humans [16, 17]. However, despite its negligible metabolism, drug interactions with fexofenadine have been reported. Coadministration of fexofenadine with ketoconazole or erythromycin resulted in increased plasma concentrations of the H_1-receptor antagonist (by 164% and 109%, respectively) [17, 18]. Since ketoconazole and erythromycin are not only inhibitors of CYP3A4, but also inhibitors of drug transporter function [16, 19], the underlying mechanism of these drug interactions appears to be inhibition of drug transporter-mediated fexofenadine elimination. Moreover, fexofenadine oral clearance was shown to be significantly increased by rifampicin [20] suggesting induction of P-glycoprotein and possibly other transport processes.

It is not clearly established whether the disposition of fexofenadine in humans is also affected by differences in P-glycoprotein function due to polymorphisms of *MDR1*. We therefore compared fexofenadine kinetics between subjects with the TT genotype of the human *MDR1* gene at position 3435 and individuals homozygous for the wild-type allele. Since several other polymorphisms have been found in the *MDR1* gene [14, 21, 22] subjects were also genotyped for nine additional variants including six leading to amino acid substitutions.

Methods

Human subjects

One hundred and four healthy Caucasian subjects living in the Stuttgart area were genotyped for the presence of the *MDR1* gene polymorphism at position 3435. Ten individuals homozygous for the wild-type allele (CC) and 10 individuals homozygous for the TT genotype participated in this study. The characteristics of the volunteers are shown in Table 1. Each subject was ascertained to be healthy by medical history, physical examination, ECG and routine laboratory testing. A negative pregnancy test result was required for the female participants. Alcohol, coffee, tea, smoking, grapefruit juice and drug intake were not permitted throughout the study beginning at 22.00 h in the evening prior to fexofenadine administration. All subjects gave their written, informed consent. The study protocol was approved by the local ethics committee (Landesärztekammer Baden-Württemberg, Stuttgart, Germany).

A sample size calculation conducted before the study indicated that the inclusion of 10 volunteers in each genotype group has a 90% power to detect 20% differences in pharmacokinetic parameters between the two genotype groups assuming that the common standard deviation is 15% of the respective means using a two group *t*-test with a 0.05 two-sided significance level. These differences are equal to or smaller than the previously observed differences in pharmacokinetics of orally administered digoxin between the two genotype groups [14].

Study protocol

After an overnight fast all volunteers received 180 mg fexofenadine HCl p.o. (Telfast® 180 mg; Aventis Pharma,

Table 1 Characteristics of 20 healthy individuals included in the study. Data (mean ± s.d.) are shown for individuals with different *MDR1* genotypes at position 2677 or 3435.

MDR1 *genotype*					
Exon 21 (G2677A, G2677T)	*Exon 26 (C3435T)*	*Subjects* n	*Sex M/F*	*Age (years)*	*Weight (kg)*
	CC	10	6/4	30.5 ± 5.9	74.4 ± 12.9
	TT	10	3/7	31.8 ± 4.7	67.4 ± 9.1
GA		1	1/0	31	96
GT		4	1/3	28.0 ± 4.6	65.3 ± 6.3
GG		8	4/4	31.6 ± 5.5	72.8 ± 11.7
TT		7	3/4	32.4 ± 5.2	68.4 ± 9.6

Bad Soden am Ts., Germany) at 08.30 h with 50 ml water. The participants abstained from food for 4 h after fexofenadine administration. Electrocardiograms and blood pressure were recorded at each time point of blood sampling. Venous blood samples (8 ml) were collected before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 7, 9, 12, 24, 30, 33, 48 and 72 h after administration of fexofenadine. Blood samples were centrifuged, and separated plasma was stored at -20° C. Urine was collected for 72 h after administration of fexofenadine. Additional blood samples were obtained for determination of P-glycoprotein function and *MDR1* mRNA expression in peripheral blood cells (see below) at the time fexofenadine was given.

Determination of fexofenadine in plasma and urine by liquid chromatography-mass spectrometry

Fexofenadine concentrations were determined by h.p.l.c. electrospray mass spectrometry as recently described by Hofmann *et al.* [23]. Briefly, 1 ml of plasma or 50 μl of urine, diluted with 950 μl of water, was mixed with internal standard (MDL 026042, kindly provided by Aventis Pharma Deutschland GmbH, Frankfurt a.M., Germany) and diluted with 1 ml of acetate buffer (0.2 M, pH 4.0). Extraction was carried out on C18 solid phase extraction cartridges as described previously [24]. The eluates were dried with nitrogen and the residue dissolved in 150 μl of the mobile phase, containing 40% acetonitrile. An aliquot of 10 μl was used for h.p.l.c. analysis. The mobile phases for h.p.l.c. were: (A) 12 mM ammonium acetate in water and (B) acetonitrile. Chromatographic separation was achieved on a LUNA CN column (2.0 mm i.d. × 10 cm, particle size 3 μm; Phenomenex, Aschaffenburg, Germany) using a linear gradient from 40% B to 60% B in 10 min. The mass spectrometer (HP 1100 MSD, Hewlett-Packard, Waldbronn, Germany) was operated in the selected ion monitoring mode using the respective MH^+ ions, m/z 502.3 for fexofenadine and m/z 530.3 for the internal standard.

Standard curves were linear in the range of 0.5–200 ng ml^{-1}. Serum samples with higher concentrations were diluted with human blank serum. Coefficient of variation (CV) of the interassay variability ($n = 14$; quality controls containing 1.0, 10 and 200 ng ml^{-1} of fexofenadine) ranged between 11.7 and 14.6%. The CV of the intra-assay variability ($n = 6$) ranged between 4.6 and 9.6%.

Genotyping

Genomic DNA from peripheral leucocytes was isolated by standard methods. Exons 2, 11, 12, 21, 26 and adjacent intronic sequences of the *MDR1* gene were amplified by PCR as previously described by Hoffmeyer *et al.* [14]. Genotyping of exon 26 was carried out by denaturing h.p.l.c. analysis [15]. Polymorphisms in exons 2, 11, 12 and 21 were detected by direct DNA sequencing, which was performed on an ABI 310 sequencer by using BigDye Terminator cycle sequencing reactions (Perkin-Elmer/Applied Biosystems, Weiterstadt, Germany).

Rhodamine efflux and quantification of MDR1 *mRNA*

To examine P-glycoprotein function in isolated peripheral blood mononuclear cells (PBMC), the extent of inhibition by low PSC-833 concentrations of efflux of the P-glycoprotein-substrate rhodamine 123 from $CD56^+$ natural killer cells was examined in comparison with rhodamine 123 efflux from $CD56^+$ cells from the same individual incubated without P-glycoprotein inhibitor as previously described by Hitzl *et al.* [15]. Briefly, after isolation of the PBMC they were incubated with rhodamine 123 (2.6 μM, Sigma, Steinheim, Germany) for 30 min. PBMC were then washed twice with PBS and incubated for another 10 min in rhodamine free medium to allow rhodamine-efflux. After washing the PBMC with PBS again they were stained with the PE-labelled CD56 antibody (Immunotech, Marseille, France). Median rhodamine fluorescence was determined in $CD56^+$ natural killer cells using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) after the efflux period and compared to median rhodamine fluorescence of cells from the same individual which were treated during the efflux period with the P-glycoprotein inhibitor PSC-833 (2.5 μM; kindly provided by Novartis, Basel, Switzerland). The individual fluorescence was calculated as percentage of the control value obtained with inhibition of P-glycoprotein by PSC-833. In addition, *MDR1* mRNA expression in PBMC was determined as described by Hitzl *et al.* [15] using a TaqMan real-time PCR assay.

Pharmacokinetic calculations

Peak fexofenadine plasma concentrations (C_{max}) and the time to maximum concentration (t_{max}) were obtained directly from the original data. The area under the plasma concentration-time curve (AUC) of orally administered fexofenadine was calculated using the trapezoidal rule. The AUC was extrapolated to infinity by dividing the last plasma concentration measured by the elimination rate constant. Elimination half-life ($t_{1/2}$) was estimated based on the terminal log-linear data points. Apparent oral clearance (CL_O) was calculated from dose/AUC$(0,\infty)$. The renal clearance (CL_R) was obtained from the equation $CL_R = Ae(0,72\ h)/AUC(0,72\ h)$, where Ae(0,72 h) represents the amount of fexofenadine excreted in urine.

The nonrenal clearance (CL_{NR}) was calculated from the equation $CL_{NR} = CL_O - CL_R$. Pharmacokinetic data were analysed using TopFit software (Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC, Gustav Fischer Verlag, Jena, Germany; Version 2.0).

Statistical methods

Data are presented as mean ± s.d. and, where appropriate, 95% confidence intervals on differences. Comparisons between the two genotypic groups were made using the unpaired *t*-test. Data from three or more different genotypic groups [e.g. exon 21 (G2677T)] were compared by analysis of variance (ANOVA) and Bonferroni *posthoc* tests. Statistical analysis was performed using GraphPad InStat software (GraphPad Software for Science Inc., San Diego, California, USA). A *P* value below 0.05 was considered to be statistically significant.

Results

The *MDR1* genotypes of the 20 participants are shown in Table 2. Overall, the subjects showed considerable variability in fexofenadine pharmacokinetics: C_{max} ranged between 241 and 1205 ng ml^{-1} (5-fold), the minimum and maximum values for AUC$(0,\infty)$ were 1579 and 8876 ng ml^{-1} h (5.6-fold) and the half-life differed nearly 3-fold within the study population (between 10 and 29 h).

The exon 26 (C3435T) polymorphism and fexofenadine disposition

Mean plasma concentration-time curves of subjects with the TT and CC genotypes at position 3435 are shown in Figure 1. The corresponding pharmacokinetic parameters are given in Table 3a. There were no statistical differences in pharmacokinetic parameters between the TT and CC group. However, compared to the CC group, subjects in the TT group showed a (non significant) trend towards higher mean AUC$(0,\infty)$ and $t_{1/2}$ values and lower mean values for apparent oral, renal and nonrenal clearance (CL_O, CL_R, CL_{NR}).

Rhodamine efflux and MDR1 *mRNA expression*

Experiments revealed a significantly lower rhodamine fluorescence in $CD56^+$ leucocytes reflecting a higher rhodamine efflux due to increased P-glycoprotein function in subjects with CC genotype in comparison with individuals with TT genotype at position 3435 (45.6 ± 7.2

Table 2 *MDR1* genotypes of all subjects.

Subject	*Intron 1 (Exon 2–1)*	*Exon 2 (A61G⋆)*	*Exon 11 (G1199A⋆)*	*Exon 12 (C1236T)*	*Intron 12 (Exon 12+44)*	*Exon 21 (G2677A⋆, G2677T⋆)*	*Exon 26 (A3320C⋆)*	*Exon 26 (T3421A⋆)*	*Exon 26 (C3435T)*
1	GG	AA	GG	CC	CC	GA	AA	TT	CC
2	GG	AA	GG	CC	CC	GG	AA	TT	CC
3	GG	AA	GG	CC	CC	GG	AA	TT	CC
4	GG	AA	GG	CC	CC	GG	AA	TT	CC
5	GA	AA	GG	CC	CC	GG	AA	TT	CC
6	GG	AA	GG	CC	CC	GG	AA	TT	CC
7	GG	AA	GG	CC	CC	GG	AA	TT	CC
8	GG	AA	GG	CT	CC	GG	AA	TT	CC
9	GG	AA	GG	CC	CC	GG	AA	TT	CC
10	GG	AA	GG	CT	CC	GT	AA	TT	CC
11	GG	AG	GG	CT	CC	GT	AA	TT	TT
12	GG	AA	GG	CT	CC	GT	AA	TT	TT
13	GG	AA	GG	CT	CC	GT	AA	TT	TT
14	GG	AG	GG	TT	CC	TT	AA	TT	TT
15	GG	AG	GG	TT	CC	TT	AA	TT	TT
16	GG	AA	GG	TT	CC	TT	AA	TT	TT
17	GG	AG	GG	TT	CC	TT	AA	TT	TT
18	GG	AA	GG	TT	CC	TT	AA	TT	TT
19	GG	AA	GG	TT	CC	TT	AA	TT	TT
20	GG	AG	GG	TT	CC	TT	AA	TT	TT

⋆ = SNPs leading to amino acid exchanges.

The positions of the identified polymorphisms correspond to positions of the *MDR1* cDNA (GenBank accession no. M14758), with the first base of the ATG start codon set to 1. SNPs that are located in introns are presented as (exon ± n), i.e. n nucleotides upstream (+) or downstream (–) of the exons that were defined by Chen *et al.* [36].

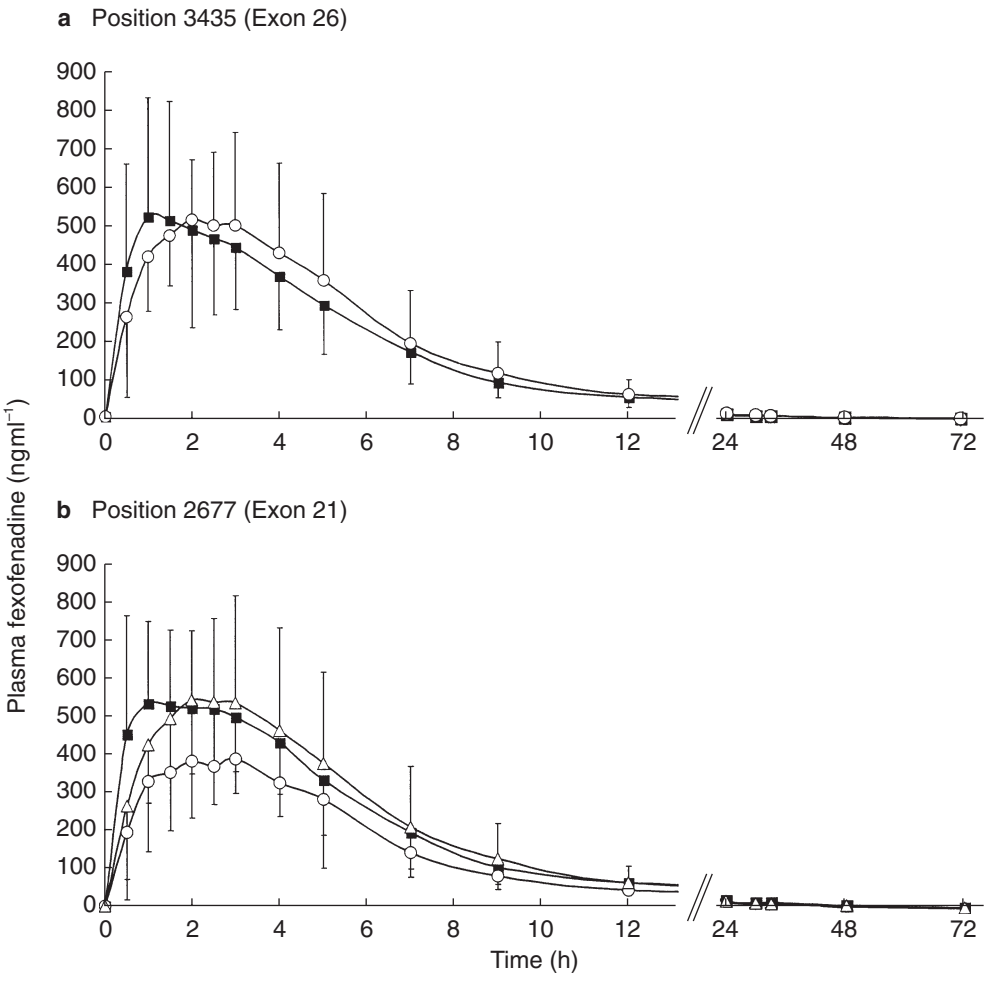

Figure 1 a) Mean plasma concentration (mean ± s.d.) time curves of orally administered fexofenadine HCl (180 mg) in 10 subjects with CC (■) and TT (○) genotypes in exon 26 of the *MDR1* gene (position 3435). b) Mean plasma concentration (mean ± s.d.) time curves of orally administered fexofenadine HCl (180 mg) in eight subjects with the GG genotype (■), four subjects with the GT genotype (○) and seven subjects with the TT genotype (△) in exon 21 of the *MDR1* gene (position 2677).

Table 3a Pharmacokinetic parameters (means ± s.d.; minimum/median/maximum for t_{max} and 95% CI on differences) after oral administration of 180 mg fexofenadine HCl to 20 healthy individuals stratified for genotypes at position 3435 of the *MDR1* gene.

	Exon 26 (C3435T)		
	CC	*TT*	*95% CI*
AUC(0,3 h) (ng ml^{-1} h)	1299.6 ± 634.9	1213.5 ± 307.8	[−580.2, 408.1]
AUC(0,∞) (ng ml^{-1} h)	3567.1 ± 1535.5	3910.1 ± 1894.8	[−1364.9, 2050.9]
t_{max} (h)	0.6/1.3/3.0	0.5/2.0/5.0	[−1.6, 0.4]
C_{max} (ng ml^{-1})	642.7 ± 308.8	620.3 ± 222.9	[−289.1, 244.3]
$t_{1/2}$ (h)	14.2 ± 5.2	16.0 ± 4.2	[−2.9, 6.5]
Ae(0,72 h) (% of dose)	9.0 ± 3.5	9.2 ± 3.5	[−3.3, 3.6]
CL_O (ml min^{-1})	957.7 ± 441.3	841.2 ± 283.3	[−483.8, 250.8]
CL_R (ml min^{-1})	74.7 ± 16.9	69.8 ± 16.0	[−11.4, 21.2]
CL_{NR} (ml min^{-1})	883.0 ± 431.3	771.4 ± 275.9	[−470.2, 246.9]

No significant differences were observed.

vs 61.1 ± 12.3%, $P < 0.05$; 95% CI 5.6, 25.5). A non-significant trend in the same direction was also observed in expression levels of *MDR1* mRNA in PBMC. Subjects homozygous for TT showed 42% lower mRNA expression levels in comparison with subjects homozygous for CC (0.7 ± 0.6 *vs* 1.2 ± 1.1, NS; 95% CI −1.3, 0.35). The G2677T polymorphism also had an effect on rhodamine fluorescence in $CD56^+$ leucocytes, although the differences did not quite reach statistical significance [GG *vs* GT *vs* TT: 45.3 ± 6.8 *vs* 57.0 ± 18.0 *vs* 60.1 ± 9.9%, $P = 0.075$; 95% CI −31.5, 8.1 (GG *vs* GT); −31.6, 1.9 (GG *vs* TT); −23.4, 17.1 (GT *vs* TT)]. No correlation was observed neither between fexofenadine AUC(0,∞) and rhodamine fluorescence in $CD56^+$ leucocytes nor between AUC(0,∞) and expression levels of *MDR1* mRNA in PBMC.

Additional MDR1 *polymorphisms and fexofenadine disposition*

Common polymorphisms in the *MDR1* gene were also found in these volunteers at positions 61, 1236 and 2677 (Table 2), with those at positions 61 and 2677 leading to amino acid changes. Therefore we determined whether these polymorphisms can have impact on fexofenadine disposition. Mean plasma concentration time curves of subjects stratified for genotypes at position 2677 (GG, GT, TT) are shown in Figure 1b and the corresponding pharmacokinetic parameters are shown in Table 3b. There were no statistically significant differences between the pharmacokinetic measurements from subjects with GG, GT and TT genotypes. No statistical differences were observed between the pharmacokinetic measurements from subjects stratified for genotypes at position 1236 (AUC(0,∞) CC *vs* TT: 3398.6 ± 1016.9 *vs* 4114.9 ± 2137.2 ng ml^{-1} h, NS; 95% CI −1250.7, 2683.3). Similarly, the A61G polymorphism did not influence fexofenadine disposition (AUC(0,∞) AA *vs* AG: 4002.2 ± 1905.7 *vs* 2947.9 ± 533.4 ng ml^{-1} h, NS; 95% CI −2966.1, 857.6).

Effect of different SNP combinations on fexofenadine disposition

Subjects were stratified for different *MDR1* genotype combinations to asses a possible combined effect of different SNPs on fexofenadine disposition. For example subjects were analysed for the SNP combination exon 12 (1236T), exon 21 (2677T) and exon 26 (3435T). The pharmacokinetic parameters did not show any statistically significant differences for this (exon 12/21/26: CC/GG/CC *vs* CT/GT/TT *vs* TT/TT/TT) and other SNP combinations.

Gender and fexofenadine disposition

Pharmacokinetic parameters from female participants did show a tendency towards higher plasma fexofenadine concentrations compared with males (AUC(0,∞): 4263.4 ± 2034.8 *vs* 3097.2 ± 930.3 ng ml^{-1} h, NS; 95% CI −459.2, 2791.7). However, when data were normalized for body weight, this trend was no longer observed (CL kg^{-1} male *vs* female: 13.2 ± 6.2 *vs* 12.9 ± 5.9 ml min^{-1} kg^{-1}, NS; 95% CI −6.2, 5.8).

Discussion

The disposition of orally administered fexofenadine revealed considerable variability among our study population (e.g. AUC(0,∞) varied 5.6-fold). *In vitro* data and results from studies with P-glycoprotein knock-out mice indicate that fexofenadine is effectively transported by

Table 3b Pharmacokinetic parameters (means ± s.d.; minimum/median/maximum for t_{max} and 95% CI on differences) after oral administration of 180 mg fexofenadine HCl to 20 healthy individuals stratified for genotypes at position 2677 of the *MDR1* gene.

	Exon 21 (G2677T⋆)			*95% CI*		
	GG	*GT*	*TT*	*GG vs GT*	*GG vs TT*	*GT vs TT*
AUC(0,3 h) (ng ml^{-1} h)	1438.8 ± 598.3	903.7 ± 361.6	1260.0 ± 350.5	[−308.32, 1378.4]	[−534.0, 891.5]	[−1219.5, 506.91]
AUC(0,∞) (ng ml^{-1} h)	3864.3 ± 1531.6	2969.1 ± 1175.2	4114.9 ± 2137.2	[−2174.3, 3964.8]	[−2844.9, 2343.6]	[−4287.7, 1995.9]
t_{max} (h)	0.6/1.5/3.0	1.5/2.5/5.0	0.5/2.0/3.0	[−3.0, 0.3]	[−1.9, 0.9]	[−0.8, 2.6]
C_{max} (ng ml^{-1})	701.9 ± 308.0	450.0 ± 127.4	663.4 ± 252.6	[−209.4, 713.1]	[−351.4, 428.3]	[−685.5, 258.7]
$t_{1/2}$ (h)	14.6 ± 5.7	15.6 ± 2.6	15.9 ± 4.7	[−9.5, 7.7]	[−8.5, 5.9]	[−9.2, 8.4]
Ae(0,72 h) (% of dose)	9.2 ± 3.6	8.5 ± 2.2	9.1 ± 4.1	[−5.6, 7.0]	[−5.2, 5.4]	[−7.0, 5.8]
CL_O (ml min^{-1})	865.8 ± 389.6	1102.8 ± 432.9	824.4 ± 302.4	[−897.4, 423.4]	[−516.8, 599.4]	[−397.6, 954.2]
CL_R (ml min^{-1})	69.2 ± 14.1	86.0 ± 19.5	63.9 ± 6.6	[−40.7, 7.1]	[−14.9, 25.4]	[−2.4, 46.5]
CL_{NR} (ml min^{-1})	796.6 ± 380.9	1016.7 ± 421.8	760.5 ± 299.4	[−867.2, 426.8]	[−510.7, 582.9]	[−405.9, 918.5]

No significant differences were observed. ⋆Data for one subject heterozygous for GA in exon 21 (2677) are not shown.

P-glycoprotein [16]. However, we did not find any statistically significant differences in fexofenadine disposition between subjects homozygous for the wild-type allele (CC) and the TT genotype at position 3435 of the human *MDR1* gene. In contrast, in the same volunteers, efflux of the P-glycoprotein substrate rhodamine 123 from $CD56^+$ natural killer cells was significantly reduced and total leucocyte *MDR1* mRNA showed a (non significant) trend towards lower values in subjects with the TT genotype at position 3435 in comparison with the CC group. The latter data are in accordance with a previous study, which found reduced intestinal P-glycoprotein levels and higher plasma concentrations after oral administration of the P-glycoprotein substrate digoxin in subjects with the TT genotype in comparison with the remainder of the population [14]. There is a significant interethnic variability in the allele frequency of the C3435T genotype [22, 25, 26]. For example, a considerably higher frequency of the CC genotype was found in Africans (83%) in contrast to Caucasians (26%) [26]. It was speculated that these interethnic differences might have implications for interethnic differences in plasma concentrations of commonly used P-glycoprotein substrates such as digoxin, cyclosporin A and HIV-1 protease inhibitors.

The question arises as to why the C3435T polymorphism was associated with increased digoxin plasma concentrations but did not contribute to the variability in fexofenadine kinetics. *In vitro* experiments and data from studies with P-glycoprotein knock-out mice clearly indicate that both drugs are effectively transported by P-glycoprotein [6, 16]. Moreover, these drugs are only metabolized to a minimal extent in humans, excluding the possibility that variable phase I or phase II biotransformation contributes to drug disposition. However, recent data indicate that the mechanisms for the uptake of digoxin and fexofenadine into cells might be different. Whereas lipophilic digoxin enters cells easily [particularly during inhibition of P-glycoprotein [19]], the uptake of fexofenadine, which exists at physiological pH as a zwitterion, into Caco-2 cells is more limited [16]. Both digoxin and fexofenadine are transported into cells by one or more members of the OATP (organic anion transporting polypeptide) uptake transporter family [16, 27]. Fexofenadine has been shown to be a substrate of the human OATP-A [16]. However, it should be noted that OATP-A expression in liver is very low. Intestinal transporters responsible for fexofenadine uptake have not yet been defined. Very recent data indicate that liver-specific OATP8 mediates the uptake of digoxin into cells [27]. Taken together, involvement of one or several uptake transporters, which might limit fexofenadine uptake *in vivo*, would provide a parsimonious explanation for the lack of association between fexofenadine kinetics and a genetic polymorphism of an efflux transporter.

Recently, it was reported that genetic polymorphisms in the *MDR1* gene are linked (1236C and 2677G and 3435C *vs* 1236T and 2677T and 3435T) and affect fexofenadine disposition in Caucasians living in the United States (with higher AUC values for individuals who carry wild-type alleles at the respective positions) [22]. These data are in contrast to the results of the present fexofenadine study in Caucasians living in Germany and to the previously shown increased digoxin plasma concentrations in subjects carrying T at position 3435 [14]. The reasons for those discrepancies are unknown. One could speculate that differences in European and North American diet (e.g. salt content [28, 29]) account for differential regulation and expression of drug transporters determining fexofenadine disposition [30, 31]. Increased fexofenadine plasma concentrations have been observed during concomitant treatment with erythromycin and ketoconazole [17, 32]. Both erythromycin and ketoconazole are not only inhibitors of P-glycoprotein function, but also of human OATP uptake transporter [16, 19]. Thus, inhibition of uptake transporter function might be an additional underlying mechanism of this and possibly other drug interactions. Similarly, reduced fexofenadine plasma concentrations were observed during coadministration of rifampicin [20]. Further studies are required to determine whether induction of OATP uptake transporters contributes, in addition to the induction, of P-glycoprotein to this drug interaction.

It should be pointed out that the highly sensitive LC-MS assay enabled us to quantify fexofenadine in plasma after administration of a single dose up to 72 h in all volunteers, which the approximately 10-fold less sensitive h.p.l.c. assays could not do [33, 34]. We were able to determine AUC (with the AUC extrapolated to infinity being less than 4% of the measured AUC) and calculate the elimination half-life, which was not altered in the group of volunteers carrying the TT genotype at position 3435. Since multiple additional polymorphisms of the human *MDR1* gene have been reported [14, 21, 22, 35], our volunteers were genotyped for nine additional polymorphisms including six leading to amino acid exchanges. It was not the primary goal of our investigation to determine the influence of polymorphisms other than that in position 3435 on fexofenadine kinetics and it needs to be emphasized that our individuals were selected for the CC and TT genotype at position 3435. Nevertheless, none of the polymorphisms investigated appeared to alter fexofenadine disposition.

In summary, in spite of *in vitro* data and results from animal experiments indicating that H_1-receptor antagonist fexofenadine is a suitable probe drug for P-glycoprotein function in humans, the present study in German Caucasian subjects indicates that *MDR1* polymorphisms do not contribute to variability in fexofenadine disposition.

Further studies are required to clarify the influence of environmental factors and possible role of uptake transporters in fexofenadine disposition.

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